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**Europäisches  
Patentamt**

Zweigstelle  
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abteilung

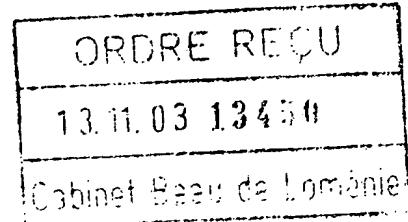
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Datum/Date  
10.11.03

Zeichen/Ref./Réf. 3J331050/2/JHD	Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°. 03291540.7-2402-
Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire Nichirei Corporation	

## COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

- Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

The following specifications given by the applicant have been approved by the Search Division:

abstract

title

- The abstract was modified by the Search Division and the definitive text is attached to this communication.

The following figure will be published together with the abstract:

1



## REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	LIU QIANG ET AL: "Overlapping PCR for bidirectional PCR amplification of specific alleles: A rapid one-tube method for simultaneously differentiating homozygotes and heterozygotes" GENOME RESEARCH, vol. 7, no. 4, 1997, pages 389-398, XP002259043 ISSN: 1088-9051 * abstract * ---	1-4	C12Q1/68
X	WO 02 057487 A (AMERSHAM BIOSCIENCES UK LTD ;KNOTT TIM (GB); SCHWARZ TEREK (UG); S) 25 July 2002 (2002-07-25) * abstract * * page 5, line 15 - page 6, line 13 * * p. 16: seq. #3, #4, #20, #21, #25 * * claims; examples 6,7 * ---	1	
X	WO 01 94638 A (APPLERA CORP) 13 December 2001 (2001-12-13) * page 29, line 3 - line 20; claims; figures * ---	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C12Q
A	WO 01 49880 A (QIAGEN GMBH ;OELMUELLER UWE (DE); KORFHAGE CHRISTIAN (DE)) 12 July 2001 (2001-07-12) * the whole document *	1-4	
A	WO 99 24452 A (LIN KUEI YING ;ISIS PHARMACEUTICALS INC (US); MATTEUCCI MARK D (US) 20 May 1999 (1999-05-20) * page 2, line 17 - page 6, line 5 * * page 25, line 9 - line 18 * * page 58, line 17 - line 25 * * page 64, line 35 - page 66, line 7; table 1 * --- --/-	1	
The present search report has been drawn up for all claims			
5	Place of search  MUNICH	Date of completion of the search  24 October 2003	Examiner  Luzzatto, E
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 03 29 1540

## DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	WO 98 45479 A (ALBANY MEDICAL COLLEGE) 15 October 1998 (1998-10-15) * claims * ----	1	
A	US 2003/039992 A1 (SCHUTT CLARENCE E ET AL) 27 February 2003 (2003-02-27) * the whole document *	1-5	
A	EP 1 138 785 A (CANON KK) 4 October 2001 (2001-10-04) * paragraph '0094!; claim 9; example 6 *		
T	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOIZUMI, TAKESHI ET AL: "Improving DNA amplification and hybridization efficiency by attaching compounds or oligonucleotide to primer" retrieved from STN Database accession no. 139:96316 XP002259044 * abstract * & JP 2003 199568 A (NICHIREI CORP., JAPAN) 15 July 2003 (2003-07-15) -----		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
5	Place of search  MUNICH	Date of completion of the search  24 October 2003	Examiner  Luzzatto, E
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 29 1540

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

24-10-2003

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 02057487	A	25-07-2002		CA 2432016 A1 EP 1352096 A2 WO 02057487 A2	25-07-2002 15-10-2003 25-07-2002
WO 0194638	A	13-12-2001		AU 6823501 A CA 2412413 A1 WO 0194638 A2	17-12-2001 13-12-2001 13-12-2001
WO 0149880	A	12-07-2001		DE 19963857 A1 WO 0149880 A2 EP 1242621 A2	26-07-2001 12-07-2001 25-09-2002
WO 9924452	A	20-05-1999		US 6028183 A US 6007992 A AU 1292899 A CA 2309340 A1 EP 1027364 A2 JP 2001522860 T WO 9924452 A2 US 2003072712 A1 US 2003176677 A1 US 6414127 B1	22-02-2000 28-12-1999 31-05-1999 20-05-1999 16-08-2000 20-11-2001 20-05-1999 17-04-2003 18-09-2003 02-07-2002
WO 9845479	A	15-10-1998		US 5994071 A AU 6949498 A EP 0975803 A1 WO 9845479 A1 US 2002076695 A1	30-11-1999 30-10-1998 02-02-2000 15-10-1998 20-06-2002
US 2003039992	A1	27-02-2003		WO 02061137 A2	08-08-2002
EP 1138785	A	04-10-2001		JP 2001275677 A EP 1138785 A2 US 2003170716 A1 US 2002197607 A1	09-10-2001 04-10-2001 11-09-2003 26-12-2002
JP 2003199568	A	15-07-2003		NONE	



This application is covered by the extended European search report pilot project at present running within the European Patent Office, applied to all European patent applications filed as first filing and searched on or after 01.07.03. Under this project the EPO issues together with the search report an opinion on whether the application and the invention to which it relates meet the requirements of the EPC. This non-binding opinion is issued free of charge as a service. This opinion may be used as the basis for an informed decision as to whether it is desired to pursue the application further or not.

For further details of this pilot project, the applicant's attention is directed to the Official Journal edition 5/2003. If any further immediate questions or comments arise the EPO Customer Services: +31-70-340 4500 or +49-89-2399 2828 can be contacted.

**The attached opinion reveals that the application or the invention to which it relates appear not to meet the requirements of the Convention (see comments on enclosed Form 2906).**

If the applicant wishes to continue with this application the examination fee must be paid. Where appropriate amendments can be filed to address the objections raised in the opinion, thus shortening the overall procedure. If no amendments are filed, the opinion will be re-issued as the first official communication under Article 96(2) and Rule 51(2) EPC.

If the examination fee has already been paid and the right to the communication under Article 96(1) EPC has been waived for this application, the first official communication under Article 96(2) and Rule 51(2) EPC will be issued promptly.



The examination is being carried out on the **following application documents**:

Text for the Contracting States:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR LI

**Description, pages:**

1-17 as originally filed

**Claims, No.:**

1-5 as originally filed

**Drawings, sheets:**

1/2-2/2 as originally filed

The following document/s (D) is/are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

- D1: LIU QIANG ET AL: 'Overlapping PCR for bidirectional PCR amplification of specific alleles: A rapid one-tube method for simultaneously differentiating homozygotes and heterozygotes' GENOME RESEARCH, vol. 7, no. 4, 1997, pages 389-398, XP002259043 ISSN: 1088-9051
- D2: WO 02 057487 A (AMERSHAM BIOSCIENCES UK LTD ;KNOTT TIM (GB); SCHWARZ TEREK (UG); S) 25 July 2002 (2002-07-25)
- D3: WO 01 94638 A (APPLERA CORP) 13 December 2001 (2001-12-13).
- D4: WO 99 24452 A (LIN KUEI YING ;ISIS PHARMACEUTICALS INC (US); MATTEUCCI MARK D (US) 20 May 1999 (1999-05-20)

1) Novelty (Art. 54 EPC)

1.1 Claims 1-3 lack novelty over D1.

D1 discloses (see abstract and p. 390, col. 1, l. 10-14) a PCR method comprising



the use of primers to which a GC tail has been added. These primers allow a high efficiency amplification and prevent the phenomenon of "megaprimering". D1, therefore, anticipates the subject-matter of claims 1-3.

- 1.2 D2 (WO-A-02057487) (see the passages cited in the Search Report) discloses the addition of fluorescent molecules such as FAM and Cy3 to the 5' end of a primer in order to increase the efficiency of amplification.  
D2, therefore, is novelty-destroying for the subject-matter of claims 1-3.
- 1.3 D3 (WO-A-0194638) describes the addition of a GC tail to a primer to increase the efficiency of an "asynchronous" PCR (p. 29, l. 3-30). It thus anticipates the subject-matter of claims 1-4.
- 1.4 No available document discloses a method for increasing the specificity of hybridisation wherein one of the compounds listed in claim 5 is added to the 5'-end of the hybridisation probe.  
The subject-matter of claim 5 is thus novel.

2) Inventive step (Art. 56 EPC)

- 2.1 D4 is considered as the closest prior art with respect to the subject-matter of claim 5. It discloses an increase in the melting temperature of the probe-target duplex ( $T_m$ ) and hence in hybridisation specificity due to the addition of specific groups to the 5'-end of an oligonucleotide (see ex. 2 and table 1).  
In view of D4, the technical problem underlying the application can be defined as that of providing an alternative method for increasing hybridisation specificity.  
The solution proposed by claim 5 is to link one of the specific groups listed in the claim to the 5'-end of the probe. This feature represents also the difference between D4 and the claimed subject-matter.  
Oligonucleotides labelled at their 5'-end with groups such as FAM or Cy3 are well known in the art (see e.g. D3, ex. 9-10). However, none of the available documents suggests using any of the groups listed in claim 5 as a means to increase  $T_m$  and thus hybridisation specificity.  
An inventive step for the subject-matter of claim 5 should thus be acknowledged.



- 3) Claim 1 lacks clarity (Art. 84 EPC) because the term "amplification efficiency" is vague and lacks a sufficiently precise technical meaning. The precise definition of this expression should be introduced into the claims (see Guidelines, C-III, 4.2), for instance based on the definition given in the description (p. 16, l. 1-7).
- 4) Concluding remarks

Should the application enter the examination phase, the following would apply:

- a) The Applicant would be requested to file new claims which take account of the above comments.
- b) **The description must be brought into conformity with the new claims to be filed;** care should be taken during revision, especially of the introductory portion including any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed, Article 123(2) EPC. To allow a speedy check as to whether the requirements of Art. 123(2) EPC are met, **the passages of the application as originally filed which are deemed to support the amendments should be clearly indicated.**
- c) The amendments should be filed by way of replacement pages, avoiding unnecessary recasting of the description. The Applicant should also take account of the requirements of Rule 36(1) EPC. In particular, fair copies of the amendments should be filed in triplicate. If handwritten amendments are submitted, they should be clearly legible for the printer.
- d) In order to expedite the procedure, the Applicant is kindly asked to clearly point out where the amendments have been made, possibly by enclosing a copy of the original pages with the corrections in manuscript.
- e) To meet the requirements of Rule 27(1)(b) EPC, the documents D1-D4 would have to be identified in the description.



- f) The amendments would have to be filed in triplicate taking into account the requirements of R. 36(1) EPC. The passages in the application as originally filed which support the amendments should be clearly indicated (Art. 123(29) EPC). To expedite the procedure, the Applicant is kindly asked to clearly indicate where the amendments have been made, possibly by filing a copy of the original sheets with the hand-written amendments.
  
- g) The Applicant's attention is drawn to the requirements of R. 29(2) EPC which allows only one independent claim per category unless the exceptional situations listed in R. 29(2) (a-c) EPC apply. Should new claims be filed, care should be taken to comply with these requirements